

where:

A_s =Area of the cefoxitin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_r =Area of the cefoxitin peak in the chromatogram of the cefoxitin working standard;

P_s =Cefoxitin activity in the cefoxitin working standard solution in micrograms per milliliter; and

d =Dilution factor of the sample.

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens*. Proceed as directed in § 436.32(b) of this chapter, using a solution containing 50 milligrams of cefoxitin per milliliter.

(4) [Reserved]

(5) *Moisture*. Proceed as directed in § 436.201 of this chapter, using the titration procedure described in paragraph (e)(1) of that section, except add about 25 milliliters of methanol in lieu of solvent A to a dry titrating vessel and proceed as directed in titration procedure 1.

(6) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams per milliliter.

(7) *Identity*. Proceed as directed in § 436.326 of this chapter, preparing the sample as follows: Prepare a solution containing about 2.5 milligrams of cefoxitin per milliliter in distilled water.

(8) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter.

[44 FR 10374, Feb. 20, 1979, as amended at 50 FR 19919, May 13, 1985; 51 FR 27532, Aug. 1, 1986]

§ 442.15 Cefixime trihydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Cefixime trihydrate is the trihydrate form of [6R-[6 α , 7B(Z)]]-7-[[[2-amino-4-thiazolyl]([carboxymethoxy]imino)acetyl]amino]-3-ethenyl-8-oxo-5-thiazabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. It is so purified and dried that:

(i) Its potency is not less than 950 micrograms and not more than 1,030 micrograms of cefixime activity per milligram, on an anhydrous basis.

(ii) Its moisture content is not less than 9.0 percent and not more than 12.0 percent.

(iii) The pH of an aqueous solution containing the equivalent of 0.7 milligram per milliliter is not less than 2.6 and not more than 4.1.

(iv) It is crystalline.

(v) The specific rotation in a 2.0 percent sodium bicarbonate solution containing 10.0 milligrams of cefixime per milliliter at 25 °C is between –75° and –88° calculated on an anhydrous basis.

(vi) It gives a positive identity test for cefixime.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results for tests and assays on the batch for potency, moisture, pH, crystallinity, specific rotation, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 10 packages, each containing approximately 500 milligrams, and 1 package containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.216 of this chapter, using an ultraviolet detection system operating at a wavelength of 254 nanometers, and a column (typically 3 centimeters \times 4.6 millimeters) packed with a 3-micron octadecyl hydrocarbon bonded silica or equivalent at ambient temperature. Reagents, working standard, test and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(A) *Phosphoric acid solution*. Add 10 milliliters of concentrated phosphoric acid to 90 milliliters of water.

(B) *Tetrabutylammonium hydroxide solution*. Dilute 25 milliliters of 0.4M tetrabutylammonium hydroxide solution to 1,000 milliliters with water. Adjust the pH to 7.0 with phosphoric acid solution.

(C) *Mobile phase*. Add 775 milliliters of the tetrabutylammonium hydroxide solution to 225 milliliters of acetonitrile.

Filter the mobile phase through a suitable glass filter or equivalent which is capable of removing particulate contamination greater than 0.5 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph.

(D) *0.1M Phosphate buffer, pH 7.0.* Add 6.8 milliliters of concentrated phosphoric acid to 300 milliliters of water. Adjust the pH to 7.0 with 10N sodium hydroxide. Dilute to 1,000 milliliters with water.

(ii) *Preparation of working standard, test and sample solutions—(A) Working standard solution.* Dissolve an accurately weighed portion of the cefixime standard with sufficient 0.1M phosphate buffer, pH 7.0, to obtain a solution of known concentration containing approximately 2 milligrams of cefixime activity per milliliter. Further dilute quantitatively to a final concentration of 0.2 milligram of cefixime activity per milliliter in 0.1 M phosphate buffer, pH 7.0. Prepare the working standard solution just prior to its introduction into the chromatograph.

(B) *System suitability test solution.* Dissolve an accurately weighed portion of cefixime working standard in distilled water to obtain a solution containing approximately 1.0 milligram of cefixime activity per milliliter. Heat this solution at 95 °C (in an oil bath) for 45 minutes. This procedure allows the (E)-isomer of cefixime to be generated *in situ*. Prepare the test solution just prior to its introduction into the chromatograph.

(C) *Sample solution.* Accurately weigh approximately 100 milligrams of the sample into a 50-milliliter volumetric flask. Dilute to volume with 0.1M phosphate buffer, pH 7.0, to obtain a stock solution containing approximately 2 milligrams of cefixime activity per milliliter. Mix well. Immediately prior to chromatography, further dilute 10 milliliters of stock solution to 100 milliliters with 0.1 M phosphate buffer, pH 7.0 to obtain a solution containing 0.2 milligram of cefixime activity per milliliter (estimated).

(iii) *System suitability requirements—(A) Asymmetry factor.* Calculate the asymmetry factor (A_s), measure data point that is 10 percent of the cefixime

peak height from the baseline, as follows:

$$A_s = \frac{a+b}{2a}$$

where:

a =Horizontal distance from point of ascent to point of maximum peak height; and
 b =horizontal distance from the point of maximum peak height to point of descent.

The asymmetry factor (A_s) is satisfactory if it is not less than 0.85 and not more than 1.5.

(B) *Efficiency of the column.* From the number of theoretical plates (n) calculated as described in § 436.216(c)(2) of this chapter calculate the reduced plate height (h_r) for the cefixime peak as follows:

$$(h_r) = \frac{(L)(10,000)}{(n)(d_p)}$$

where:

L =Length of the column in centimeters;
 n =number of theoretical plates; and
 (d_p) =Average diameter of the particles in the column in micrometers.

The absolute efficiency (h_r) is satisfactory if it is not more than 15 for the cefixime peak.

(C) *Resolution.* The resolution (R) between the peak for cefixime and the peak for the (E)-isomer of cefixime (generated *in situ*) is not less than 1.1.

(D) *Coefficient of variation (relative standard deviation).* The coefficient of variation (S_R) in percent) of five replicate injections is satisfactory if not more than 2.0 percent

(E) *Capacity factor (k).* Calculate the capacity factor (k) for cefixime as follows:

$$(k) = \frac{t_r - t_m}{t_m}$$

where:

t_r =Retention time of solute; and
 t_m =Retention time of solvent or unretained substance, calculated as follows:

$$t_m = \frac{(3.1416)(D^2)(L)(0.75)}{4F}$$

where:

D =Column diameter in centimeters;
 L =Column length in centimeters;

0.75=Average total column porosity; and
F=Flow rate in milliliters per minute.

The capacity factor (*k*) for cefixime is satisfactory if it is not less than 5 and not more than 11.

If the system suitability requirements have been met, then proceed as described in § 436.216(b) of this chapter. Alternate chromatographic conditions are acceptable provided that the system suitability parameters are met. However, the sample preparation described in paragraph (b)(1)(ii)(C) of this section should not be changed.

(iv) *Calculations.* Calculate the micrograms of cefixime anhydrous free acid per milligram as follows:

$$\frac{\text{Micrograms of cefixime per milligram}}{= \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}}$$

where:

A_u=Area of the cefixime peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the cefixime peak in the chromatogram of the cefixime working standard;

P_s=Cefixime activity in the cefixime working standard solution in micrograms per milliliter;

C_u=Milligrams of sample per milliliter of sample solution; and

m=Percent moisture content of the sample.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 0.7 milligram per milliliter.

(4) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(5) *Specific rotation.* Dissolve and dilute an accurately weighed sample with sufficient 2 percent sodium bicarbonate to obtain a concentration of approximately 10 milligrams of cefixime per milliliter. Proceed as directed in § 436.210 of this chapter, using a 1.0-decimeter polarimeter tube. Calculate the specific rotation on the anhydrous basis.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using a potassium bromide disc containing 0.5 percent of cefixime. Dissolve 5 to 6 milligrams of cefixime in 2 milliliters of methanol. Triturate to insure solution.

Evaporate the solvent to dryness and using the dried sample, prepare the potassium bromide disc.

[53 FR 24257, June 28, 1988; 53 FR 26712, July 14, 1988; 54 FR 47205, Nov. 13, 1989]

§ 442.16 Cefprozil pentahydrate.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Cefprozil pentahydrate is pyridinium, 1-[[7-[(2-amino-4-thiazolyl)-[1-carboxy-1-methylethoxy]imino]acetyl]-amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-, hydroxide, inner salt, [6*R*-[6*α*,7*β*(*Z*)]]-, pentahydrate. It is so purified and dried that:

(i) Its potency is not less than 950 micrograms and not more than 1,020 micrograms of cefprozil activity per milligram on an anhydrous basis.

(ii) Its loss on drying is not less than 13.0 percent and not more than 15.0 percent.

(iii) The pH of an aqueous solution containing 5 milligrams of cefprozil per milliliter is not less than 3.0 and not more than 4.0.

(iv) It is crystalline.

(v) It gives a positive identity test for cefprozil.

(vi) Its high molecular weight polymer content is not more than 0.05 percent.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, crystallinity, identity, and high molecular weight polymer content.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay—(1) Potency.* Proceed as directed in § 442.16a(b)(1).

(2) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 5 milligrams of cefprozil per milliliter.